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with the tissue-specific auxiliary subunits, previously known as four β-subunits (β1 - β4). We previously identified a leucine-rich repeat (LRR) containing protein, LRRC26, as a new type of BK channel auxiliary subunit, which causes an unprecedented large negative shift in voltage dependence of channel activation. Here we report a group of LRRC26 paralogous proteins LRRC52, LRRC55 and LRRC38 that potentially function as LRRC26-type auxiliary subunits of BK channels. LRRC52, LRRC55 and LRRC38 produce marked shifts in the BK channel's voltage dependence of activation in hyperpolarization direction to different extents. They together with LRRC26 show distinct expression in different human tissues and may have a broad influence of the BK channel function in different tissues or cell types.

2093-Plat
A Modular Heat-Sensing Domain Activates K_{2P}2.1 through a Heat-Insensitive Gate
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Ion channels often have modular structure with dedicated gating and sensory domains. Ligand binding to a localized site in the sensory domain usually affects channel function through allosteric effects on the gate. It is not clear whether this paradigm is applicable to ion channels regulated by temperature. Indeed, temperature may have an effect on the whole channel structure and thus, bypass the need for a dedicated heat-sensing domain. Heat leads to activation of the 'leak' two-pore (K_{2P}) potassium channel K_{2P}2.1 (KCNK2/TREK-1) through opening of a selectivity filter-based outer gate in the extracellular pore domain. We sought to investigate whether the pore plays a role in sensing heat or whether it only functions as a gate that responds to the commands from the temperature-sensing elements located elsewhere in the channel. We found that the incorporation of mutations designed to prevent allosteric communication between the intracellular C-terminal domain and the pore domain of K_{2P}2.1 resulted in channels that remain functional but fail to respond to temperature. These results indicate that the gating mechanism of the pore lacks intrinsic temperature sensitivity and that the heat-sensing elements of K_{2P}2.1 are confined within its C-terminus. Our data provide experimental support for the general notion of the existence of modular temperature sensing domains and highlight functional distinction between gating and sensory elements in heat-sensitive ion channels.

2094-Plat
K2P1 Assembles with K2P3 or K2P9 to Form SUMO-Regulated Task Background Channels in Cerebellar Granule Neurons
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Cell-surface K2P1 channels are most often electrically quiet despite their widespread expression in excitable tissues. Previously, we studied human K2P1 expressed in Chinese hamster ovary cells and found one small ubiquitin-like modifier protein (SUMO) conjugated to one K2P1 subunit to be sufficient to silence the dimeric channels (Plant et al., 2010, PNAS 107). Here, we assess K2P1 sumoylation in cultured rat cerebellar granule neurons (CGN). K2P channels have been posited to produce IKso (the standing outward potassium current responsive to changes in pH and volatile anesthetics) in CGN. First, we developed a fluorescent method to count single mRNA transcripts and showed that those for KCNK1, KCNK3 and KCNK9 (encoding K2P1, K2P3 and K2P9, respectively) were present together in individual CGNs at a ratio of 2:8:1. Next, using antibody-mediated FRET, we observed native K2P1, K2P3 and K2P9 to interact with SUMO1 at the neuronal surface whereas native K2P2 (TREK1) did not. Unlike channels with K2P1, the function of channels with K2P3, K2P9 or K2P2 were found to be insensitive to SUMO1. We reconciled the apparently discrepant findings by showing that K2P1 subunits are incorporated into mixed assemblies with K2P3 or K2P9 (but not K2P2) to form novel two P domain, acid-sensitive (TASK) channels. Channels with just one K2P1 subunit were held in silent reserve at the CGN surface by sumoylation. Accordingly, intracellular application of the desumoylating enzyme SENP1 to CGN doubled the magnitude of IKso (decreasing excitability via expected shifts in resting membrane potential and resistance) and tripled the response to halothane. Because K2P1, K2P3 and K2P9 are expressed together throughout the body and the SUMO pathway is ubiquitous this mechanism of regulation is expected be common.

2095-Plat
Activity-Dependent Transcriptional Regulation of M-Type K^+ Channels by AKAP79/150-Mediated NFAT Actions
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M-type K^+ channels, encoded by the KCNQ2-5 family of genes, play key roles in the regulation of neuronal excitability; however, less is known about the mechanisms controlling their expression. Here we propose that neuronal stimulation induces elevated M-channel expression by activation of NFAT transcription factors in response to Ca^{2+}/calcineurin (CaN)-mediated dephosphorylation, orchestrated by A-kinase-anchoring protein (AKAP) 79/150. We observed augmented mRNA for KCNQ2-3 and currents after stimulation of rat sympathetic neurons by either high-K^+ or acetylcholine (ACh), by qRT-PCR and perforated-patch whole-cell clamp. Stimulation also elicited an increase in intracellular [Ca^{2+}] and nuclear translocation of both endogenous and transfected GFP tagged-NFATc1-c2 from the cytoplasm to the nucelus. As evidence that this regulation of M channel expression is NFAT-mediated, exogenous expression of constitutively-active NFAT in sympathetic neurons increased tonic M-channel expression, which was not further increased by high-K^+ stimulation. Moreover, this augmented transcription was suppressed by incubation of the neurons with the CaN inhibitor, cyclosporine A, and the membrane-permeable VIVIT inhibitory peptide, which competes with CaN for binding to NFATs. The involvement of AKAP79/150 in CaN/NFAT regulation of M-channel expression was also studied in sympathetic neurons isolated from AKAP150+/+ and AKAP150-/- mice. We found AKAP150-/- neurons to lack NFATc1 nuclear translocation and augmented expression of M channels after high-K^+ stimulation, which was "rescued" by transfection of WT AKAP79 or a non-PKC binding AKAP79 mutant, but not AKAP79 mutants unable to bind CaN. Removal of external Ca^{2+} or addition of nifedipine during stimulation eliminated NFATc1 nuclear translocation and augmented M current, suggesting the role of L-type channels as the activity sensor. Thus, neuronal-activity regulates M-channel transcription, which in turn controls neuronal excitability. This activity-dependent transcriptional regulation of M channels involves a Ca^{2+}-dependent NFAT signaling pathway, which requires AKAP79/150 targeting of CaN.

2096-Plat
Calcium Binding Proteins (CaBPs) Selectively Modulate Ca_V1 versus Ca_V2 Channels
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Calcium-binding proteins (CaBPs) comprise a subfamily of calmodulin (CaM)-like neuronal Ca^{2+}-binding proteins that are widely expressed throughout the brain. Intriguingly, CaBPs have been reported to blunt the CaM-mediated Ca^{2+} feedback regulation in multiple subtypes of Ca_V1, and even Ca_V2 channels (*Nat Neurosci* **5**:210). This modulation of Ca^{2+} feedback potentially influences numerous neurobiological functions, thus motivating ongoing investigation of the mechanism of CaBP effects. Here, in the course of discerning the structural determinants of CaBP operation, we uncovered striking selectivity in the actions of CaBPs for Ca_V1, but not Ca_V2 channels. While mouse mCaBP4 eliminates Ca^{2+}/CaM-mediated inactivation (CDI) of Ca_V1 channels (confirming reports of multiple groups), we now find no such effect on both Ca^{2+}/CaM-dependent facilitation (CDF) of $Ca_V2.1$ channels and CDI of $Ca_V2.1$ and $Ca_V2.3$ channels. Indeed, further analysis revealed the structural basis for such selectivity. Systematic FRET 2-hybrid assays revealed a bevy of CaBP4 binding sites on various intracellular regions of $Ca_V1.3$. As such, we made chimeric channels using $Ca_V2.3$ as the backbone, and substituting intracellular loops from $Ca_V1.3$ to identify functionally relevant mCaBP4 sites. Transfer of the $Ca_V1.3$ carboxyl terminus was sufficient to confer mCaBP4 modulation on $Ca_V2.3$ channels, but CDI was only partially blunted by mCaBP4. By contrast, substitution of the amino-terminus, III-IV loop, and carboxyl-terminus of $Ca_V1.3$ into $Ca_V2.3$ resulted in complete elimination of CDI by mCaBP4. These results suggest that mCaBP4 acts in concert through multiple sites to fully eliminate CDI. Because mouse mCaBP4 induces the most potent effects, we emphasized characterization with this particular CaBP, though other CaBPs may reproduce the same trends. The remarkable ability of CaBPs to selectively modulate only Ca_V1 but not Ca_V2 channels opens new avenues for customization of neurobiological effects, and for development of selective pharmacology targeting Ca^{2+} regulation.

Platform: Membrane Structure II

2097-Plat
Resolving the Structure of Ordered Domains in Single Hydrated Lipid Bilayers by New X-Ray Diffraction Methodology
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Saturated lipids spontaneously form rigid domain in lipid membranes. X-ray scattering from these domains indicate that their sizes vary between 2-60nm